## AMENDMENTS

**In the Specification:**

**A. The last full paragraph on page 1 has been amended to recite:**

~~Alloderm™ and Integra™~~ ALLODERM™ and INTEGRA™ human skin substitutes are two currently popular examples of human skin substitute commercially available in the market. ~~Integra™~~ INTEGRA™ artificial skin, a brand of artificial skin is sold by Integra LifeScience Corporation of New Jersey, USA, and has been approved by FDA for use in the USA since 1996. Artificial skin is a bilayer biosynthetic sheet comprising porous collagen-glycoaminoglycan integrated with a thin silicone membrane as an outer layer. The use of artificial skin such as ~~Integra~~ INTEGRA™ artificial skin as a biocompatible acellular dermal replacement in deep and full-thickness burn wounds is well known.

D1

**B. The paragraph bridging pages 1 and 2 has been amended to recite:**

It has been observed that within about 14 to 21 days following the grafting of ~~Integra~~ INTEGRA™ artificial skin, there is full vascularization of the neodermis formed in the ~~Integra~~ INTEGRA™ artificial skin. Thereafter an ultra thin split thickness skin graft must be harvested from a donor site in order to cover the neodermis immediately after the silicone membrane is removed. Substantial research effort has been undertaken in the past to determine the possibility of reliably grafting CEA on the neodermis, since an effective combination of CEA and ~~Integra~~ INTEGRA™ artificial skin should eliminate the second operative stage, the associated pain and scaring, as well as a need for a second donor site, which may not be available in extensively burned patients. If the grafted CEA does not 'take' on the neodermis of ~~Integra~~ INTEGRA™ artificial skin after the silicone membrane is peeled off, it can be replaced by another CEA. Whereas, in the conventional application of ~~Integra~~ INTEGRA™ artificial skin, another split thickness autograft must be harvested from a second or even a third donor site. There have been

D2

very limited initial anecdotal reports on experience with such a combination technique, such as Sheridan *et al.* 1999 and Pandya *et al.* 1998. At the 10th Congress of International Society For Burn Injuries, November 1998 in Israel, the difficulties with the conventionally cultured graft anchoring onto the neodermis of ~~Integra~~ INTEGRA™ artificial skin were addressed. The exact reasons for such difficulties remain unknown.

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**C. The first full paragraph on page 2 has been amended to recite:**

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~~Laserskin~~™ LASERSKIN™ artificial skin material is a thin and pliable biosynthetic membrane comprising a 100% benzyl esterified hyaluronic acid derivative suitable for use as a substratum in the growth of skin cells. The recommendation of the manufacturer is to seed human keratinocytes on ~~Laserskin~~ LASERSKIN™ artificial skin preseeded with irradiated 3T3 cells as feeder layer. When following the manufacturer's recommendation, it was found that, after the initiation of the formation of keratinocyte colonies, the xenogenic 3T3 cells growing on the ~~Laserskin~~ LASERSKIN™ artificial skin were less likely to be washed away than those growing on a culture dish as in the conventional Green's method during each flushing procedure with phosphate-buffered saline. It is believed that the remaining 3T3 cells or debris might have sensitized the host to xenogenic antigen resulting in undesired late graft rejection. What is needed is a cultivation and engraftment procedure with a biocompatible, durable human skin substitute.

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**D. The first paragraph of the Summary on page 4 has been amended to recite:**

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According to the invention, autologous cultured keratinocytes grown on a biocompatible substratum are engrafted on the neodermis of artificial skin covering a wound. Autologous keratinocytes may be cultivated on a commercially available membrane such as ~~Laserskin~~™ LASERSKIN™ artificial skin (available from Fidia Advanced Biopolymers Ltd., Abano Terme (PD), Italy) following pre-seeding with autologous or allogenic dermal fibroblasts. The resultant composite material may then be

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applied on the neodermis of artificial skin which had been previously engrafted on the patient. The composite material, and specifically Composite Biocompatible Skin Graft (CBSG) material comprises autologous keratinocytes and allogenic or autologous dermal fibroblasts grown on the substratum. A method for fabricating the composite material includes the application of dermal fibroblasts onto the substratum as a feeder layer and then inoculating autologous keratinocytes on the resultant structure. A method for engraftment comprises first applying an artificial skin with a protective silicone membrane on a wound area, thereby allowing vascularization; following vascularization, removing the silicone membrane and engrafting the cultured composite material onto the vascularized artificial skin.

D4 cont

**F. The second full paragraph on page 5 has been amended to recite:**

CBSG material according to the invention offers notable advantages. First, the basal proteins (including the early basement membrane proteins such as collagen IV and fibronectin) of the cultured graft are protected from dispase treatment because the keratinocytes are directly cultivated on a pliable ~~Laserskin~~ LASERSKIN™ artificial skin. This is believed to enhance anchorage of the cultured keratinocytes on the neodermis of ~~Integra~~ INTEGRA™ artificial skin. Second, in addition to acting as a feeder layer, the dermal fibroblasts in the inventive CBSG material evidently produce a number of proteins such as native collagen fibers and fibronectin which is believed to facilitate the attachment of a cultured graft. Third, the cultured keratinocytes of the inventive CBSG can be grafted five to seven days sooner than can traditionally-cultured keratinocytes. This is because cultured keratinocytes of the inventive CBSG are capable of being transferred and grafted at the sub-confluent or less differentiated stage rather than at a later confluent stage. Fourth, since there is minimal need for a donor site there is less likelihood of widespread scarring related to donor site harvesting. Fifth, the cultured keratinocytes of CBSG can be handled much more easily than the conventional CEA during its application on the neodermis of the artificial skin. Fewer cultured cells are lost or damaged during the transfer and application of CBSG. This should improve the

D5

D5 cont

success rate of the cultured graft. Sixth, the inventive engraftment technique can result in higher demand and broader scope of clinical applications for artificial skin.

**G. The heading and paragraph between lines 11 and 19 on page 7 has been amended to recite:**

**~~Laserskin~~ LASERSKIN™ artificial skin**

~~Laserskin~~ LASERSKIN™ artificial skin is a biosynthetic biocompatible substratum for keratinocyte cultivation according to the invention. It is a form of thin and pliable biosynthetic membrane comprising a 100% benzyl esterified hyaluronic acid derivative. ~~Laserskin~~ LASERSKIN™ artificial skin is commercially available from Fidia

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Advanced Biopolymers Ltd., Abano Terme (PD), Italy. ~~Laserskin~~ LASERSKIN™ artificial skin was found to be useful to the inventors' experiments, although its preparation and application are not done according to the manufacturer's conventional instructions. There is nothing to preclude the use of compatible bioequivalents or human skin substitutes would also work in a similar fashion with the inventive CBSG.

**J. The paragraph bridging pages 9 and 10 and the first three paragraphs of page 10 have been amended to recite:**

**Results**

Twenty-four hours after the seeding on the CBSG substratum, the non-viable and unseeded keratinocytes were carefully washed away and concentrated for counting. There were no detectable 3T3 cells or fibroblasts detached from the ~~Laserskin~~ LASERSKIN™ artificial skin during the washing procedure. The seeding efficacy of keratinocytes was calculated as:

D7

$$\frac{[\text{Total keratinocytes count (before seeding)} - \text{non-seeded keratinocytes}]}{\text{Total keratinocytes count (before seeding)}} \times 100\%$$

It is evident that the selected type of CBSG substratum provided a suitable culture template for the in vitro proliferation of keratinocytes. On a plain ~~Laserskin~~

LASERSKIN™ artificial skin substratum, human keratinocytes showed a mean seeding efficacy of 75%. These human keratinocytes had a 95% seeding efficacy on ~~Laserskin~~ LASERSKIN™ artificial skin populated with human fibroblasts and a 98% on 3T3 cell-seeded ~~Laserskin~~ LASERSKIN™ artificial skin (Table 1). Rat keratinocytes had a seeding efficacy of 36% on plain ~~Laserskin~~ LASERSKIN™ artificial skin. The respective seeding efficacies of rat keratinocytes on 3T3/~~Laserskin~~ LASERSKIN™ artificial skin and on allogenic fibroblasts/~~Laserskin~~ LASERSKIN™ artificial skin were 91% and 88%. The seeding efficacies of human/rat keratinocytes growing on 3T3 cell/ ~~Laserskin~~ LASERSKIN™ artificial skin or on allogenic fibroblasts/~~Laserskin~~ LASERSKIN™ artificial skin were significantly ($p < 0.001$) better than those seeded on plain ~~Laserskin~~ LASERSKIN™ artificial skin, as noted in the Fisher Exact Testing using Stat Xact (version 2.05) statistical package.

D7 cont

It is believed that human/rat fibroblasts could achieve a role similar to that of the 3T3 cells in enhancing the seeding efficacies of keratinocytes growing on ~~Laserskin~~ LASERSKIN™ artificial skin with respective p values of 0.445 and 0.646 using the same statistical package.

The optical transparency of the ~~Laserskin~~ LASERSKIN™ artificial skin allowed regular inspection of the grafted wound bed as it healed. Skin biopsies were taken from the center of the grafted area of the subject. It was observed that the polypropylene ring prevented the migration of epithelium from the wound edge as no epithelial cell was found in the control rat wound sutured with polypropylene ring alone up to day 21. In sixteen out of the twenty (80%) animal wounds covered with Type A CBSG, the keratinocytes formed a multi-layered epithelium that had a basal layer in contact with underlying connective tissue. The undersurface of epidermis did not show rete. Fibrovascular ingrowth of connective tissue into the ~~Laserskin~~ LASERSKIN™ artificial skin was observed. Eight (40%) of the Type B CBSG sites and seven (35%) of the Type C CBSG sites showed re-epithelization. Histologically there was no observable

D7 cont

epithelium in 3 (15%), 10 (50%), and 12 (55%) of the grafted CBSG sites of Types A, B and C respectively (Table 2).